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2,4-Dinitrophenol: a novel activating reagent in nucleotide synthesis via the phosphoramidite route. Design of new effective phosphitylating reagents†

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Abstract

2,4-Dinitrophenol (DNP) is a remarkably efficient activator in the reaction of P(III) amides with nucleosides to give P(III) esters in excellent yield. Typical examples of this novel procedure are presented herein. Mechanistic features of the activation were elucidated by model studies of the reaction of bis(2-cyanoethyl)diisopropylphosphoroamidite with benzyl alcohol. The importance of the initial protonation stage and formation of an intermediate P(III)-2,4-dinitrophenyl ester were clearly demonstrated by ^{31}P NMR spectroscopy and independent synthesis. New phosphitylating reagents containing the 2,4-dinitrophenoxy group do not require any activation.

Keywords: nucleosides; nucleotides; phosphoroamidites; 2,4-dinitrophenol; synthesis.

Phosphoroamidites are among the most important reagents in the synthesis of phosphates of biological interest by the 'phosphite approach'. Tetrazole, tetrazole salts with amines and structural analogues of this heterocycle are generally used as activators for the reaction between alcohols and phosphoramidites. Tetrazole, the most widely employed activator, must often be used in excess and be of high purity, which involves hazardous sublimation.[1] Other activators like amine hydrochlorides and acyl chlorides have been used less frequently.[2] In spite of impressive progress in this field there are still important problems to be solved. When the necessity arises to produce biophosphates in large quantities, better procedures are required for activation of phosphoramidites.

In our studies on the synthesis of modified nucleosides we faced the challenge of finding an alternative mode of activation which did not use tetrazole or similar compounds. Recently we have found that trimethylchlorosilane, Me_3SiCl, is a remarkably good activator in the reaction of phosphoroamidites with nucleosides.[3]

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† This paper is dedicated to the memory of Professor Alexander Krayevsky.

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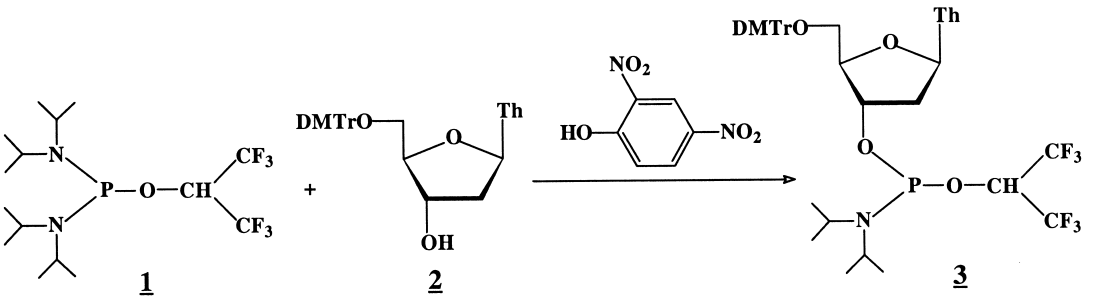
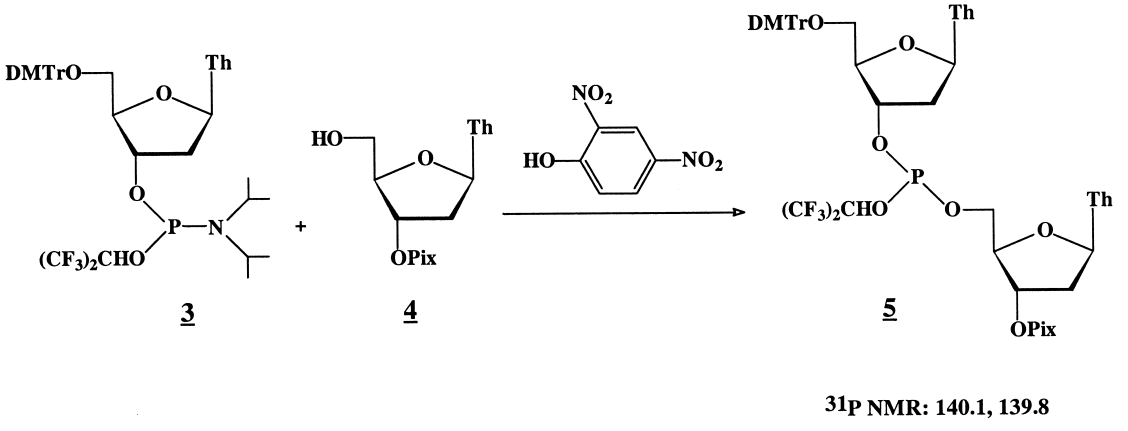
In a search for other effective 'non-classical' activators we turned our attention to strongly acidic phenols. We have found that 2,4-dinitrophenol (DNP), which with a pK_a value of 4.1 is similar to that of tetrazole (pK_a = 4.9), acts as an efficient activator of phosphate synthesis via the phosphoroamidite procedure. The reaction of P(III) amidites with an equivalent amount of nucleoside in the presence of DNP proceeds in very high yield and at rates comparable or higher than those when tetrazole is used. Phosphitylations activated by DNP take place at room temperature in solvents like THF, CH_2Cl_2 or MeCN. On average the amount of activator required for efficient coupling is ca. 1.5 equivalents of the stoichiometrical ratio.[4] Most of our experiments were performed with P(III) amides derived from diisopropylamine in order to conform with the most popular phosphitylation procedures. Selected examples illustrating our methodology are chosen from nucleotide chemistry. DNP is distinctly superior to tetrazole when P(III) amides bear a strongly electron-attracting group at the phosphorus centre.[5]

The coupling of amidite **1** prepared according to Tataku et al.[6] with 5′-DMTr-thymidine **2** proceeds in a chemoselective way according to Scheme 1 to give the amidite **3** (98% yield as determined by ^{31}P NMR). In this and all other cases, no removal of DMTr and Pix (9-(9-phenyl)xanthenyl) protective groups is observed.



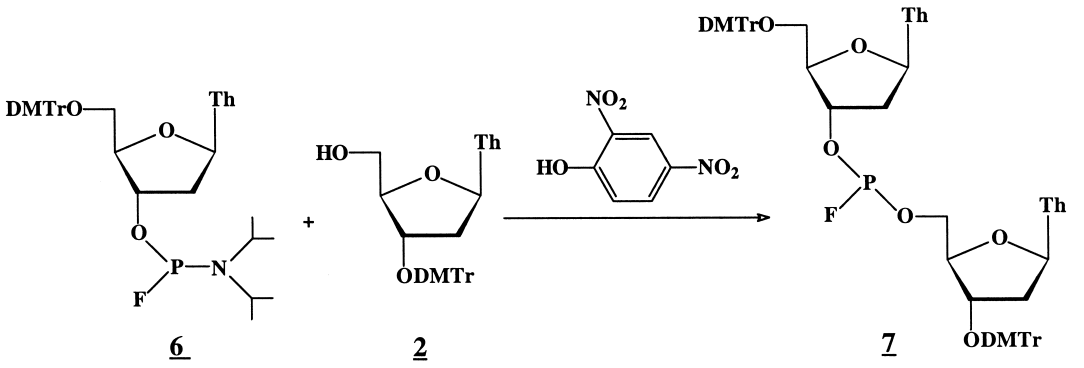
Scheme 1.

The amidite **3** when allowed to react with 3′-pixyl-thymidine **4** gives the dinucleoside phosphite **5** (95% yield as determined by ^{31}P NMR) (Scheme 2).



Scheme 2.

The fluoroamidite **6** prepared in our earlier studies[7] reacts with 3′-DMTr-thymidine **5** in the presence of DNP yielding the dinucleoside phosphorofluoridite **7** in excellent yield (Scheme 3). This compound is identical with samples prepared by us with the aid of other activators.[7]



6 **2** **7**

^{31}P NMR: 162.9, 149.1; 162.7, 148.9

^{19}F NMR: -73.4, -79.3; -74.0, -80.0

J_{PF}= 1116.9 Hz., 1115.6 Hz.

^{31}P NMR: 139.7, 124.6; 138.4, 123.4

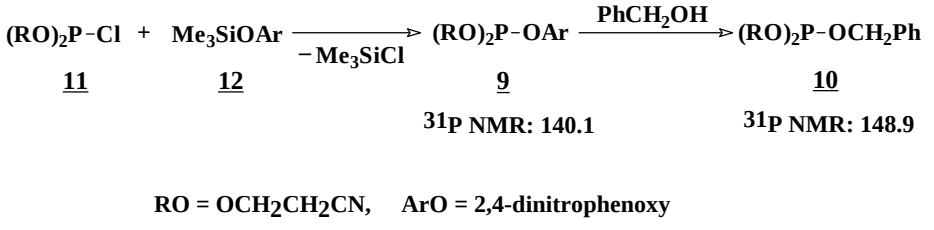
^{19}F NMR: -53.6, -60.1; -53.9, -60.2

J_{PF} = 1224.0 Hz., 1220.7 Hz.

Scheme 3.

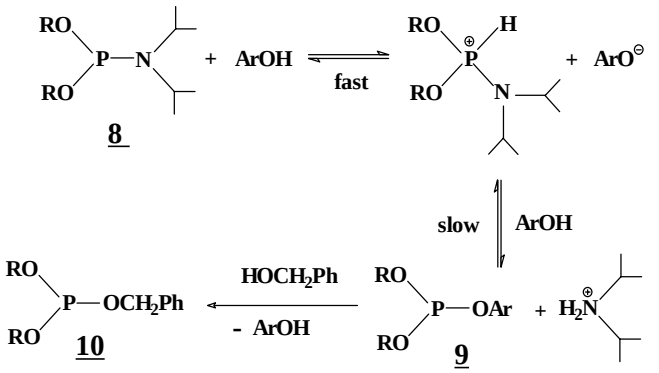
When pure diastereoisomers of **6** were used[8] the coupling of Scheme 3 proceeds in a non-stereoselective way giving a (1:1) mixture of the corresponding diastereoisomers of the fluoroamidites **7** (98% yield as determined by ^{31}P NMR).

The mechanism of activation by DNP is presumed to involve its reaction with P(III) amidite in a similar manner to that accepted for activation in the presence of tetrazole.[9] We anticipated formation of the intermediate bearing the 2,4-dinitrophenoxy group attached to the P(III) center. This assumption was confirmed by a model experiment involving the reaction of bis(2-cyanoethyl)-*N*,*N*-diisopropylphosphoroamidite **8** and benzyl alcohol in the presence of 1.5 equivalents of DNP. Formation of the intermediate **9** (^{31}P = 140.1 in C_6D_6) en route to the formation of benzyl-bis-(2-cyanoethyl)phosphite **10** was observed by ^{31}P NMR spectroscopy. In order to verify the structure of the intermediate **9**, its independent synthesis was carried out according to Scheme 4 from the chloridite **11** and trimethylsilyl 2,4-dinitrophenol **12**. The phosphite **9** prepared in this way reacts spontaneously with benzyl alcohol to give the phosphite **10** (97% yield as determined by ^{31}P NMR).



Scheme 4.

The mechanistic features of the DNP activation are shown in Scheme 5.

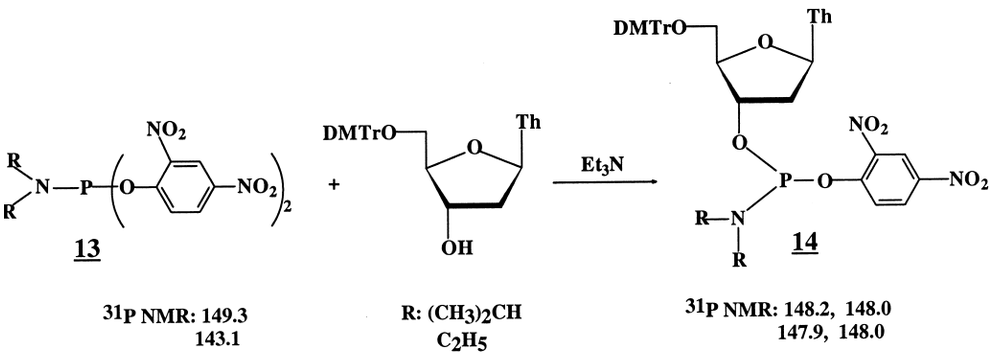


Scheme 5.

The signal intensity of **9** increases with increasing amount of DNP. Further evidence for the mechanism described in Scheme 5 is that addition of triethylamine dramatically reduces the rate of formation of the phosphite **10**. This inhibition provides evidence for reversible acid catalysis. The nucleophilic catalysis step is slow and cannot explain the observed inhibition. The practical consequence of this study is that DNP must be used in at least an equimolar amount. The fact that the reaction described in Scheme 3 proceeds in a non-stereoselective way is explained by an epimerization at the chiral phosphorus atom due to exchange of ligands with the excess DNP present in the reaction medium. This situation is similar to that observed for the activation of phosphoramidates by tetrazole. In conclusion, two important factors are involved in catalytic properties of DNP: acidity and the ability to form reactive intermediates.

A useful consequence of these studies is the possibility of designing new phosphitylating reagents which react spontaneously with alcohols without any activation. For example bis(2,4-dinitrophenyl)phosphoramidite **13** was prepared by a standard procedure from the corresponding aminodichlorophosphine. Amide **13** reacts with nucleosides in a non-selective way and a mixture of products is formed. From a mechanistic point of view this result is consistent with spontaneous displacement of a 2,4-dinitrophenoxy group which liberates free DNP. The latter activates the amido group leading to further ligand exchange. However, if the phosphitylation by **13** is performed in the presence of one mole of triethylamine, high chemoselectivity is observed, as shown in Scheme 6. The amidate **14** is formed in good yield (92% yield as determined by ^{31}P NMR).

DNP proved to be an excellent activator for the hydrolysis of phosphoroamidates to yield the corresponding hydrogen phosphonates. For example: from the amidite **3** the corresponding H-phosphonate diester was obtained in very high yield as a result of hydrolytic replacement of the amido group.



Scheme 6.

Acknowledgements

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References

1. Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859; Uhlman, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543; Beaucage, S. L.; Iyer R. P. *Tetrahedron* **1993**, *48*, 2223.
2. Gryaznov, S. M.; Letsinger R. L. *Nucleic Acids Res.* **1992**, *20*, 1879; Karl, R. M.; Richter, W.; Klösel, R.; Mayer, M.; Ugi, I. *Nucleosides Nucleotides* **1996**, *15*, 379.
3. Dabkowski, W.; Tworowska, I.; Michalski, J.; Cramer, F. *Chem. Commun.* **1997**, 877.
4. Commercial wet DNP was dissolved in an excess of toluene and the toluene–water azeotrope was removed by distillation. The residual solution was evaporated in vacuo and the solid DNP kept for 24 h in vacuo in a vessel containing P_2O_5. All procedures involving activation by DNP must be performed under anhydrous conditions. *Typical procedure:* A solution of DNP (15 mmol) in dry CH_3CN (10 ml) was added dropwise to a solution of the corresponding phosphoroamidite (**1**, **3**, or **6**) (10 mmol) and nucleoside (**2** or **4**) (10 mmol) in dry CH_3CN (20 ml) at room temperature under a nitrogen atmosphere. After 2 h the mixture was evaporated to dryness and the resulting residue was purified by column chromatography using CH_2Cl_2:$CH_3C(O)CH_3$ (10:1 v/v) as eluent to give the corresponding pure phosphoridites **3** (90% yield), **5** (92% yield) or **7** (90% yield).
5. The compounds described in Schemes 1–6 gave correct data in FAB-mass spectroscopy and their identity was confirmed by ^1H, ^{31}P, ^{13}C and ^{19}F NMR spectroscopy. Some diagnostic spectral data are included in Schemes 1–6.
6. Tataku, H.; Watanabe, T.; Hamamoto, S. *Tetrahedron Lett.* **1988**, *29*, 81.
7. Dabkowski, W.; Tworowska, I. *Tetrahedron Lett.* **1995**, *36*, 1095.
8. Dabkowski, W.; Tworowska, I.; Michalski, J.; Cramer, F. *J. Chem. Soc., Chem. Commun.* **1995**, 1435.
9. Dahl, B. H.; Nielsen, J.; Dahl, O. *Nucleic Acids Res.* **1987**, *15*, 1729; Berner, S.; Mühlegger, K.; Seliger, H. *Nucleic Acids Res.* **1989**, *17*, 853.